An in vitro-in vivo taste assessment of bitter drug: Comparative electronic tongues study

Journal:	*Journal of Pharmacy and Pharmacology*
Manuscript ID:	JPP-14-0230.R1
Wiley - Manuscript type:	Research Paper
Date Submitted by the Author:	n/a
Complete List of Authors:	Maniruzzaman, Mohammed; University of Greenwich, School of Science Douroumis, Dennis; University of Greenwich, Medway School of Science
Keywords:	In vivo/in vitro Correlation < Biopharmaceutics and Drug Disposition, Pharmaceutical Analysis, Pharmaceutics and Drug Delivery
Abstract:	Objectives The efficiency of the Astree e-tongue and Taste Sensing system TS5000Z for the evaluation of the taste masking effect of hot melt extruded formulations was investigated in this study. Methods Hot melt extrusion (HME) processing was optimized using Randcastle single screw extruder (USA) to manufacture extrudates with desirable characteristics. Cationic model drug propranolol HCl (PRP) was processed with the anionic polymers - Eudragit L100® (L100) and Eudragit L100-55 (Acryl-EZE). In vitro taste masking efficiency of the two polymers was performed by using two different e-tongues (Astree e-tonge and TS5000Z). Key Findings Both e–tongues were able to detect the taste masking variations of the extrudates and were in good agreement with the in vivo results obtained from a panel of six healthy human volunteers (R2>0.84). However, each e-tongue sensor demonstrated different sensitivity suggesting a careful consideration of the experimental findings during melt extrusion is necessary for the development of taste masked formulations. Furthermore, FT-IR spectroscopy and NMR studies revealed possible drug polymer intermolecular interactions as the mechanism of successful taste masking. Conclusions HME can effectively be used to manufacture taste masked extruded formulations while both e-tongues demonstrated satisfactory taste analysis for the development of taste masked formulations.

An *in vitro-in vivo* taste assessment of bitter drug: Comparative electronic tongues study

Mohammed Maniruzzaman[1*] and Dennis Douroumis[1]

[1] School of Science, University of Greenwich, Central avenue, Chatham Maritime, Chatham, Kent, ME4 4TB, UK

[*] To whom correspondence should be addressed: Mohammed Maniruzzaman, University of Greenwich, Medway School of Science, Chatham Maritime, ME4 4TB, Kent, UK, email: M.Maniruzzaman@gre.ac.uk. Phone: 0044(0)208 331 8342, Fax: 0044 (0) 208 331 9805.

Abstract

Objectives The efficiency of the Astree e-tongue and Taste Sensing system TS5000Z for the evaluation of the taste masking effect of hot melt extruded formulations was investigated in this study.

Methods Hot melt extrusion (HME) processing was optimized using Randcastle single screw extruder (USA) to manufacture extrudates with desirable characteristics. Cationic model drug propranolol HCl (PRP) was processed with the anionic polymers - Eudragit L100® (L100) and Eudragit L100-55 (Acryl-EZE). Solid state of the drug in polymer matrices was evaluated by scanning electron microscopy (SEM), differential scanning calorimetry (DSC), particle size analysis, Fourier Transform Infra-Red (FT-IR) and Nuclear Magnetic Resonance (NMR) analysis. *In vitro* taste masking efficiency of the two polymers was performed by using two different e-tongues (Astree e-tonge and TS5000Z). The results obtained from both e-tongues were further compared and contrast to find out the sensor outputs in all formulations.

Key Findings Solid state analysis of the extruded formulations revealed the presence of amorphous PRP. Both e–tongues were able to detect the taste masking variations of the extrudates and were in good agreement with the *in vivo* results obtained from a panel of six healthy human volunteers (R^2>0.84). However, each e-tongue sensor demonstrated different sensitivity suggesting a careful consideration of the experimental findings during melt extrusion is necessary for the development of taste masked formulations. Furthermore, FT-IR spectroscopy and NMR studies revealed possible drug polymer intermolecular interactions as the mechanism of successful taste masking.

Conclusions HME can effectively be used to manufacture taste masked extruded formulations while both e-tongues demonstrated satisfactory taste analysis for the development of taste masked formulations.

Keywords: Taste masking, Propranolol HCl®, Eudragit L100®, Acryl-EZE, Astree E-Tongue, TS5000Z.

Introduction

Masking the bitter taste of active pharmaceutical ingredients (APIs) is considered a major challenge especially for the development of orally administered dosage forms in pharmaceutical industry. [1, 2] Due to the unpleasant sensation bitter taste is always the least desired and sometimes completely undesired option whereas sweet taste is pleasant for most of the people regardless their age and origin. [3] In reality most of the APIs used in oral drug products have a bitter taste which is not only undesirable but also frequently has a negative influence on the palatability of the final dosage forms. For paediatric population unpalatable/bitter taste is the most challenging as children are highly sensitive to taste and actively refuse the unpalatable drugs. [4, 5] It is often impossible to block bitter taste receptors (due to their increased numbers) from the drug without compromising the mechanism of its action. [6, 7] The extent of taste masking of an API depends almost exclusively on the type of formulation (solid or liquid). Being the first preference, commercial oral liquid dosage forms contain artificial sweeteners (e.g saccharin and aspartame) and flavours to mask the tastes which are often limited due to the regulatory requirements. Due to very poor effects of this method and possibilities of toxic and allergic reactions, European Medicines Agency (EMA) strongly recommends another way for taste masking instead of adding sweeteners or flavours. [8] An ideal solution for this problem should involve the prevention of any contact of the unpalatable APIs with the taste buds without the addition of taste additives. Such an ideal formulation can be developed by applying an appropriate coating or encapsulation on the API or via manufacturing solid dispersions [9, 10] in inert matrices (polymeric/ lipidic). The coated/ encapsulated drug then can be dispersed in water.

In vivo taste masking evaluation studies are performed by healthy human volunteers and involve taste assessments based on the individual scores. [11] A well-established statistical method is required to overcome errors and variability between volunteers within the limit of threshold taste perceptions. According to the FDA guidelines studies on paediatric formulations should not be performed on paediatric volunteers due to ethical conflicts. On the other hand in order to design paediatric formulations mature volunteers should also be prohibited due to large physiological differences of taste sensation. [7] Ethical doubts on experiments in children and difficulties with interpretation of the results eventually indicate the need to use alternative *in vitro* methods for taste evaluations. [4,7] In the last few years, electronic tongues (e-tongues) became popular for the evaluation of the *in vitro* taste performance for repeatable analysis of pharmaceutical products. [12, 13] Electronic tongues are sensor array systems which are able to determine single substances as well as complex mixtures of various substances. Electronic -tongue is a device simulating human sense of

taste that allows for the identification and classification of liquid samples. Regardless of the selected chemical compounds e-tongues work to the recognition of general sample properties such as specific taste (e.g bitter). They consist of an array of chemical sensors and a pattern recognition system. [14-16] Over the last few years electronic tongue systems have taken the advantage of different measuring principles including potentiommetry, voltammetry and amperometry. Currently, there are commercially available e-tongues which have successfully been employed for taste assessments in various pharmaceutical formulations. Astree e-tongue (Alpha MOS, France) and taste sensing system TS-5000Z (INSENT, Japan) are two common e-tongues which have been used as *in vitro* taste assessment tools. [17-21]

The aim of this novel study is the evaluations of the taste masking efficiency of hot melt extruded formulations of bitter API (PRP) by using two different e-tongues (Astree e-tongue and TS-5000Z) simultaneously and studying the mechanism of the effective taste masking via extrusion processing.

Materials and methods

Materials

Propranolol HCl (PRP) was purchased from Sigma Aldrich (London, UK). Eudragit L100 (L100) and Eudragit L100-55 (Acryl-EZE) was kindly donated by Evonik Pharma Polymers (Darmstadt, Germany) and Colorcon ltd respectively. The HPLC solvents were of analytical grade and purchased from Fisher Chemicals (UK). All materials were used as received.

Preparation of formulation blends and hot-melt extrusion (HME) processing

PRP formulations with L100 and Acryl-EZE to be extruded were mixed properly in 100g batches for 10 min each. A Turbula (TF2, Basel) mixer was used to blend the powder formulations (drug/polymers ratio used were 10:90 w/w). Extrusion of all PRP formulations were performed using a Randcastle single-screw extruder (RCP 0625, USA) equipped with a 5 mm rod die using 100°C/113°C/155°C/155°C/155°C (Feeder to die) temperature profiles. The screw speed maintained for all extrusion was 15rpm. The produced extrudates (strands) was grinded by using a Ball Milling system (8 balls, 1.5 cm diameter) to obtain granules (<500 µm). Grinding by ball milling was carried out with a rotational speed of 400 rpm for 5 min.

Particle morphology and size distribution

Scanning Electron Microscopy (SEM) was used to study the surface morphology of the extrudates. Samples were mounted on an aluminum stage using adhesive carbon tape which was then placed in a low humidity chamber prior to the analysis. Samples were also coated

with gold–palladium, and microscopy was performed using Cambridge Instruments - S630 (Cambridge, UK) operating at an accelerating voltage of 5 kV. All samples were run singlet.

The particle size distribution of the micronized granules of all formulations was measured by dry sieving. The method involved stacking of the sieves on top of each other and then placing the test powder (50 g) on the top sieve. The nest of sieves was subjected to a standardized period of agitation (20 min) and then the weight of the material retained on each sieve was accurately determined to give the weigh percentage of powder in each sieve size range. All samples were run triplicate.

Differential scanning calorimetry (DSC) and Modulated temperature DSC analysis

DSC runs of pure actives, physical mixtures and extrudates were carried out using a Mettler-Toledo 823e (Greifensee, Switzerland) differential scanning calorimeter. Sealed aluminium pans were used to prepared sample weighing about 2-5 mg of samples (n= 3). Samples were heated at 10°C/min heating rate from -40 to 220°C. At the time of heating, samples were under nitrogen atmosphere. In addition modulated temperature differential scanning calorimetry (MTDSC) studies were performed from 25°C to 250°C with an underlying heating rate of 1°C/min. The pulse height was adjusted to 1-2°C with a temperature pulse width of 15-30 s.

***In vivo* taste masking evaluation**

In vivo taste masking evaluation of pure API, polymers and all active extruded formulations was performed in accordance to the Code of Ethics of the World Medical Association (Declaration of Helsinki). [19] Six (6) healthy volunteers of either sex (age 18–25) were selected (Male = 3, female = 3) from whom informed consent was first obtained (approved by the Ethics Committee of the University of Greenwich, Ref: UG09/10.5.5.12). All volunteers were also trained prior to the experiment. The equivalent of 100 mg of pure PRP or PRP based extrudates (containing equal amounts of API) were held in the mouth for 60 seconds and then spat out. The selection of samples was random and in between of two samples analysis mineral water was used to wash each volunteer's mouth. The bitterness was recorded immediately according to the bitterness intensity scale from 1 to 5 where 1, 2, 3, 4 and 5 indicate none, threshold, moderate, bitter and strong bitterness. All samples were evaluated in triplicate.

***In vitro* taste masking evaluation: Astree E-Tongue (Alpha MOS)**

The assays were better performed on Astree e-tongue system equipped with an Alpha M.O.S. sensor set #2 (for pharmaceutical analysis) composed of 7 specific sensors (ZZ, AB, BA, BB, CA, DA, JE) on a 48-positions autosampler using 25 ml beakers. Acquisition times were

fixed at 120s. [19] All the data generated on Astree system were treated using multidimensional statistics on AlphaSoft V12.3 software. Each solution was tested on Astree e-tongue at least 3 times. 3 replicates were taken into account for the statistical treatment. The average values of all sensors signals between 100 and 120 s constitute the raw data for later multivariate statistical data processing. This processing allows to map the data on 2-dimension maps (Principal Components Analysis - PCA, Discriminant Factorial Analysis, Statistical Quality Control, Partial Least Square, etc). With Astree e-tongue, PCA was used to evaluate the differences and similarities between various samples or groups of samples. The samples are represented in a two-dimensional or three-dimensional space with reference to the selected components (PC1 and PCn). The components are classified according to the level of information they produce. Astree sensors were cleaned up with deionised water between each sample measurement.

Sample preparation for Astree E-Tongue

In vitro taste masking evaluation was carried out with an Astree E-Tongue equipped with 7 different sensor sets. To be as close as panellists taste's conditions, each drug was diluted for 60s under magnetic stirring in 25 ml of deionised water to reach API concentration corresponding to a final dose of 100 mg. Then solutions were filtered (as the particles can damage the ASTREE sensors and thus alter the quality of results) with Buchner funnel fitted with filter paper at 2.5µm pore size (Table 1). Analysis for each API was done in triplicate.

***In vitro* taste masking evaluation: TS-5000Z sensing system**

The assays were realized on TS-5000Z taste sensing system equipped with a BASIC sensor set (for pharmaceutical analysis) which are suitable for basic APIs composed of 10 specific sensors (AAE, CT0, CA0, C00, AE1, AC0, AN0, BT0, GL1) on a 48-positions autosampler using 25 ml beakers. Each measurement cycle was consisted of measuring a reference solution (Vr) followed by sample solution (Vs) and then the aftertaste (Vr) followed by a cleaning procedure. The ‘‘aftertaste’’ was measured by determining the change in membrane potential caused by the adsorption of the analyte to the lipid membrane. Sensor outputs for both taste (also called relative value (R)) and ‘‘aftertaste’’ were then calculated in relation to the initially determined sensor response to the reference solution (Vr). [1, 22] Acquisition times were fixed at 120s with a BT0 negatively charged sensor. All the data generated on TS-5000Z system were treated using multidimensional statistics. Each solution was tested on TS-5000Z at least 4 times and triplicates were taken into account for the statistical treatment. Sensors were then cleaned up in references solutions (30 mM KCl + 0.3 mM tartaric acid) between each sample measurement. The samples were dissolved in 50 mL of 10 mM KCl aq.

solutions and further diluted to prepare 0.03, 0.1, 0.3, and 1 mM solutions as standards. Then solutions were filtered with Buchner funnel fitted with filter paper at 2.5µm pore size (n=3).

FTIR spectroscopy

FT-IR analysis was performed on the drug, polymer, drug/polymer physical mixtures, and extrudates using Perkin Elmer PE1600 (Massachusetts 02451 USA) Fourier Transform Infrared Spectra according to the KBr disc method from 400 – 3600 wavelength/cm^{-1} range (n=3).

Nuclear magnetic resonance (NMR) studies

NMR spectra were recorded on a Jeol ECA 500 NMR spectrometer, incorporating a 5mm inverse probe (The ^{1}H operating frequency was 500 MHz). ^{1}H NMR spectra of the drugs, polymers and drug/polymer formulations were recorded using the standard Jeol pulse sequence. All samples were dissolved in CD_3OD, degassed and then maintained at 25^{o}C during data acquisition. Samples were referenced with respect to the solvent. The solution concentration of the drug was 2mg/ml, the polymers were 18 mg/ml, and the drug/polymer formulation was 20mg/ml (the overall drug content in the formulations was 10%) (n=3). ^{1}H T_1 relaxation experiments were recorded for all samples using a standard inverse recovery experiment. Recovery delays (τ) were investigated between 10 ms and 20 s. The relaxation delay was set to be >5T_1. T_1s were calculated from curve fitting and peak intensities which were obtained from the spectra recorded for different recovery delays. Jeol, curve fitting software was utilized during this process.

Statistical analysis

All data generated and collected during *in vitro* taste analysis by both the e-tongue and taste sensing system TS-5000Z were treated by statistical methods. Results were expressed as raw data in mV of the sample relative measurement to the reference. Sensor signal results were evaluated via multivariate data analysis. Multivariate analysis, such as principal component analysis (PCA), was used to reduce the multidimensional space without losing information. Using PCA, the most abundant information contained in the original data could be transformed into the first principal component (PC-1), and the second most abundant information is transformed into the second component (PC-2). For multivariate data analysis, raw data were pretreated by mean centring and scaling to unit variance. Data processing, graphical illustration and statistical interpretation of the results were carried out using Excel 2010 (Microsoft, Redmond, WA, USA). INSENT and Alpha MOS software.

Results and discussion

Hot-melt extrusion process: Particle morphology and size distribution

Extrusion processing of all PRP based formulations was performed at 155°C with relatively lower screw speed of 15 rpm in order to allow homogenous blending of the drug/polymer binary mixtures. The rationale underlying selecting high processing temperature was due to the higher Tgs of polymeric carriers used. Various formulation trials were conducted at the optimization stage ranging a drug loading 10-20% (w/w ratios). But keeping the final dose in the finished product e.g. tablets in account, 10% (w/w) drug loading was chosen to proceed with. Preliminary results showed no significant differences in terms of the solid state of the extrudates and physical performance between the formulation containing 20% PRP and 10% PRP. Another reason underlying the selection of PRP- a cationic charged substance, as a model drug and two different polymers (anionic charged) as carriers, was to possibly facilitate an intermolecular interactions in order to mask unpleasant taste of the bitter API. Theoretical miscibility parameter calculations showed that the solubility parameter of PRP (21.94 $MPa^{1/}2$) calculated by Van Krevelen equation is quite close to that of L100 (22.75 $MPa^{1/}2$) and Acryl-EZE (21.65 $MPa^{1/}2$). [6] It has been reported in previous studies that if the difference of the solubility parameters between drug and polymer is less than $7MPa^{1/2}$, then the polymer is likely to be miscible with the API to form an amorphous solid dispersions. [6] As a result the cationic PRP may interact with the functional groups of the negatively charged polymers to effectively mask bitter taste of the drug.

SEM was used to examine the surface morphology of the drug and extrudates. The extrudates containing L100 and Acryl-EZE showed homogenous particles distribution on the extrudates surface with PRP (Fig. 1) indicating excellent HME processing of the extruded materials to form solid dispersions. The particle size distribution depicted in Fig. 1 shows particle sizes lower than 500 µm for most formulations ranging from 40 – 400µm. A small percentage can be seen at sizes <40µm as the milling process was optimized to reduce fines in the final extruded batches.

Solid state analysis

DSC was conducted in order to analyze the solid state (crystalline or amorphous) of the pure drug, polymers, drug/polymers binary mixtures and drug/polymer extrudates. The thermal transition of PRP in Fig. 2 showed an endothermic peak corresponding to its melting point at 166.65°C (ΔH= -126.25 J/g). The bulk polymers showed Tgs at 83.97°C and 164.83°C corresponding to Acryl-EZE and L100, respectively (Fig. 2). A sharp melting peak was also observed in the Acryl-EZE thermogram at 59.2°C (data not shown), corresponds to the

presence of crystalline plasticizers in the co-processed formulation.[19] MTDSC analysis of the binary physical blends of PRP/L100 and PRP/Acryl-EZE exhibited PRP endothermic peaks shifted at slightly lower temperatures of 162.41°C to 153.62°C indicating a drug/polymer interaction at small extent. The same shift at lower temperatures was also observed for the Tgs of the polymers at 73.16°C and 98.82 °C for PRP/Acryl- EZE and PRP/L100, respectively.

Furthermore, the extruded PRP/Acryl-EZE (and L100) extrudates exhibited single glass transition peaks at 63.36 and 74.84°C, respectively which indicates the presence of drug/polymers miscibility and formation of molecular solid dispersions. When the two components are miscible the Tg of the extruded sample lies between the Tgs of the individual components (amorphous drug and polymers) according to Gordon – Taylor equation. [6, 19] The Tg of PRP was determined at 34.74°C (data not shown). DSC analysis confirmed the presence of molecular dispersions in all extruded formulations.

Previous studies [6] showed that the diffraction patterns of both PRP physical mixtures exhibited crystalline peaks with reduced intensities corresponding to pure drug. The diffractograms of the extruded formulations were characterized with the absence of drug intensity peaks indicating amorphous or molecularly dispersed state.

***In vivo* taste masking**

The masking efficiency of the developed granules was evaluated *in vivo* (approved by University of Greenwich, UK ethics committee) with the assistance of six healthy human volunteers (age 18 – 25). The statistical data collected from the *in vivo* study for the pure active substance and the extruded formulations are depicted in Fig. 1. The data analysis showed significant suppression ($p < 0.05$) of the bitter taste for the API. These results demonstrate the influence of the polymeric carriers and importance of drug loading in the final formulation. Both polymers showed effective taste masking capacity with descending order L100> Acryl-EZE. Furthermore, the HME formulations presented excellent masking effect for active concentrations (10%) of the API. This could be due to the possible drug polymer interactions in the solid dispersions manufactured during extrusion process. In the solid dispersions cationic active substance (PRP) may have interacted with the functional group of the negatively charged polymers. These interactions facilitated a hydrogen bonding interaction between the active amide group of API and carboxylic group of polymers and consequently masked the bitter taste of the active. A similar study has also been reported elsewhere. [2] In Fig. 3 the sensory data obtained from the panelists interestingly showed that the taste masking efficiency of L100 is not similar to that of Acryl-EZE for the API used. This could be attributed to the pH dependant dissolution properties of Acryl-EZE (pH ≥ 5.5)

compared to that of L100 (pH ≥ 6) as the saliva represents a basic pH (~7.4) in healthy individuals. However, the sensory scores of the API in different formulations are within the range (below 2) which has been demonstrated as optimum by *in vitro* evaluations. [1, 11]

In vitro taste evaluations (Astree e-tongue)

Astree e-tongue was used for *in vitro* taste analysis of the drug and active formulations. Principal Component Analysis (PCA) associated to complementary data processing was used. [20] Based on the statistical analysis taste maps were constructed in order to determine the distances between active and polymer solutions. Actually the distance between each active formulation and its corresponding placebo is indicative of how close or how far the taste of the two samples is. The interpretation of the taste maps suggests that the shorter the distance (Euclidean distance) between active and placebo (polymer), the better the taste masking of the active ingredient. Thus the distance between any drug polymer pairs in the taste maps is indicative of the taste masking efficiency of the extruded polymer formulations from which the estimated Discrimination Index (DI in %) can be determined for each solution. This indicator (DI) takes into account the average difference between the pairs (i.e drug and active formulation or polymer and active formulation) to compare the dispersion or taste masking effect. It is assumed that the higher the DI values (maximum 100%), the longer the distance between groups and the lower the masking effects.

In Fig. 4a, the taste map shows significant discrimination between placebo and active solutions with PRP. Liquid sensors were able to detect the presence of the drug in the extruded formulations. Considering the pure drug in deionized water the extrudates with L100 (10% drug loading w/w) shows a better taste improvement compared to that of Acryl-EZE (Fig. 4a). The distance between the placebo and the active formulations indicates the efficiency of the taste masking of the active by both polymers. The observed distance proximity between extrudates of PRP and placebo is noticeable (for an example, 19% taste improvements of PRP with L100). This trend is likely to be linked with a pH influence of Acryl-EZE in deionized water (pH ~5.5) which leads to a higher separation of placebo from the active formulations by dissolving faster than L100 (pH > 6.0). From the PCA graphs it can be seen that the placebo, the API, and the extrudates are discriminated which means significant taste differences.

Based on the Astree e-tongue experimental results it was also possible to design the DI graphs for the drug – polymer combinations. In Fig. 4b, it can be seen that the distance between active and placebo formulations with Acryl-EZE (DI 62%) is higher than that of

L100 (DI 40%) in the extrudates, indicating better taste masking efficiencies of L100 (19% taste masking/improvement) than Acryl-EZE polymeric system.

However, this was expected as the use of deionized water was intentionally selected in order to test the sensitivity of the Astree e-tongue in variations of the drug dissolution rates. As mentioned above the polymers dissolve in different pH which results faster drug release for Acryl-EZE compared to L100.

Sensory correlated models based on Partial Least Square (PLS) were built to evaluate the correlation with sensory scores. The correlation model is considered as valid and fits with panel perception (R^2>0.80). But it should be taken with care as all data on sensory tests (number of panelists, variability on measurement) were not communicated. It's quite obvious from the Fig. 4c that the *in vitro* taste assessment studies carried out with Astree e-tongue correlated very well with the *in vivo* panelists data (R^2 = 0.9892 (Acryl-EZE); 0.9959 (L100)).

PRP was found to be quite bitter by the panelists (sensory score 5) similar to the Astree e-tongue evaluation. The impact of Acryl-EZE carrier was negligible as Acryl-EZE itself was also found not to be bitter (sensory score 1). Contrary, PRP/L100 formulations demonstrated improved taste masking even though the bulk polymer showed threshold-moderate bitterness. However, the PLS were in good agreement by complementing the *in vivo* study, where the panelists recorded a moderate taste with L100 (and no taste with Acryl-EZE).

A further statistical analysis was performed by considering the standard deviations (SD) and therefore the relative standard deviations (RSD) of all extruded formulations. The findings of the standard deviations studies for bulk drugs and the extrudates are summarized in Table 2. It can be seen that the statistical analysis of SD and RSD for all formulations, showed positive results towards effective taste masking of bitter PRP. It is accepted that the scale of interpretation measure is SD < 50: Fair and SD < 30: good, respectively. Based on this scale of interpretation, the results showed (Table 2) that the calculated mean SD values for all formulations is ≤ 13 which suggests good taste masking of the API in the extruded formulations.

INSENT TS-5000Z sensing system

The *in vitro* masking effect of the extruded formulations in artificial saliva was also evaluated by using the INSENT TS-5000Z e–tongue. The distance percentages (%) between active substances and formulation solutions were estimated in four different time intervals (0.5 min,

1 min, 10 min and 30 min) as they are indicative of taste masking efficiency of the extruded formulations. In addition, the discrimination index (DI, %) was determined for each solution. Initial trials showed that the BT0 sensor of TS-5000Z system responded to the DPD and PRP at the each concentration ranging from 0.03, 0.1, 0.3 and 1 mM. Therefore, BT0 sensor can be useful for detecting bitterness of the API in the concentration ranging from 0.03 to 1 mM.

In contrast with the Astree e-tongue, in INSENT TS-5000Z system, the lower DI values the longer the distance in taste responses between the pairs (drug and formulations) and thus a higher discrimination, which means greater masking effect. The DI (%) values can help to assess the significance of difference between the formulations. In Fig. 5a the bitter taste suppression of PRP in the L100 extrudates is quite significant even after 30 min as the DI index (%) is only about 60% while after 1 min DI is 40% (DI index (%) close to 0% indicates no taste). In contrast the PRP/ Acryl-EZE extrudates (Fig. 5b) did not show taste suppression similar to the L100 polymer but still the DI index (%) estimated by the BT0 sensor around 98% after 30 min and 85% in 1 min, respectively was considered effective (but less than L100).

The normalized taste graphs showed significant discrimination between all active formulations and active ingredient solutions (Fig. 5a-b) suggesting lower taste masking efficiency of Acryl-EZE for the API compared to the taste suppression of L100.

As mentioned before, liquid sensors are able to detect the taste of the drug in the masked formulations (up to 0.3mM API), therefore the *in vitro* taste masking results detected by the BT0 sensor are quite sensitive and consistent. For that reason, in comparison with the pure drug in the reference solutions (artificial saliva) the extrudates exhibited taste masking effects. As mentioned above this could be attributed to the pH dependency of both polymers which present different drug release due to their different pH values. However the e-tongue sensor did perceive the taste of bitter APIs from the dissolved polymer matrices.

Sensory correlated models were built to evaluate the correlation with sensory scores. The correlation model was considered as valid and fitted with panel perception (Fig 5c) and complemented the sensory findings from the panelists' scores to conclude the statement that L100 has better taste masking efficiency than Acryl-EZE. The TS-5000Z taste sensing system demonstrated different sensitivity to each sample with high correlation (R^2=0.94) to the taste scores, suggesting that the sensor responds selectively according to bitterness intensity by providing quantitative information.

The BT0 sensor was also used to determine the taste of pure polymers. Interestingly as shown in Fig. 5d it was not possible to detect any taste for both L100 and Acryl-EZE.

Apparently the contribution of the bulk polymers was not taken in account as INSENT uses a different approach compared to that of Astree e-tongue.

Fourier Transform Infra-Red (FT-IR) analysis

FT-IR has been used to study interactions in drug/polymer dispersions by providing valuable information regarding the oppositely charged ionic drug/polymer interactions at molecular level. [23] By showing the appearance of additional bands, alterations in wavenumber position or broadening of functional groups compared to the spectra of the pure drug and polymer the FTIR spectra gives an indication of drug/polymer interactions. The FTIR spectra for the extruded formulations are shown in Fig.6.

The characteristic bands of CO- vibrations of the carboxylic acid groups in L100 and Acryl-EZE are shown at ~1705 cm^{-1} and of the esterified carboxylic groups at ~1735 cm^{-1}. The FTIR spectra of the PRP extrudates in comparison with the pure materials are depicted in Fig. 6, which showed a new absorption band at ~1560 and ~1555 cm^{-1} for PRP/ L100 and PRP/ Acryl-EZE, respectively. This is considered to be the result of the presence of amine group alongside the carboxyl group in the solid dispersions. [24] During the FTIR process the resonance is possible between the two CO-bands within COO- groups. As a result, the characteristic CO- absorption is replaced by the band of auto-symmetrical vibrations of the COO- group in the 1555- 1560 cm^{-1} region of the FTIR spectra [24, 25] which belongs to the polymer (L100 or Acryl-EZE). This type of spectra changes provides strong evidence of strong interactions between the anionic methacrylate polymers (-COO) and the cationic PRP (amine group) by enabling the formation of hydrogen bonds with the amine group of the drug.

NMR analysis

^{1}H T_1 NMR spectroscopy was employed to monitor the possible chemical changes at the molecular level by analyzing chemical shifts of NMR signals. Such a change has been observed in regards with the chemical shifts in the ^{1}H NMR spectra of drug and drug/polymer solutions. [26, 27] Previous studies showed that NMR analysis carried in DMSO successfully revealed possible drug/polymer interactions in molecular level. Initially solid state NMR was conducted in order to elucidate possible drug/polymer interactions; however the low drug loading in our formulations didn't accord NMR a meaningful interpretation.

^{1}H T_1 NMR experiments were used to analyse spin relaxation times. Different relaxation rates of nuclear spins can be related to aspects of molecular structure and additionally to internal molecular motion. The reasoning behind these experiments was to

look at potential changes of the drug's molecular motion, before and after the extrusion. Indeed, it would be assumed that the free drug (with a low molecular weight) would have quite a high molecular motion leading to fairly high T_1 relaxation delays. After formulation, any consequence of an interaction between the drug and polymer would result in a decrease in the amount of molecular motion observed for the drug. It can be seen in Fig. 7 that the T_1 relaxation times have significantly been decreased in all PRP/L100 formulations. About 16-20 folds of decrease in the T_1 relaxation times have been observed in the extruded formulations. This significantly indicates that the free drug (with a low molecular weight) had quite a high molecular motion leading to fairly high T_1 relaxation delays (times) while the extruded formulations showed very low T_1 relaxation delay. This was due to the strong drug/polymer interactions leading to a significant decrease in the relaxation time. T_1 relaxation delays are particularly sensitive to intermediate molecular motions which result in short T_1s. Molecules which have fast or slow molecular motion can have comparable T_1s. [6]

This NMR analysis indicates the presence of molecular interaction between the drug and polymers in solutions, although the type of the interactions cannot be elucidated. In addition, the presence of such intermolecular interaction can contribute to the possible taste masking mechanism of all drug/polymer combinations during extrusion.

Conclusions

In this study the performance of two e-tongues was evaluated for the development of taste masked PRP formulations processed by hot melt extrusion. The optimized formulations were also evaluated *in vivo* by panellists and showed very good masking efficiency. Both e-tongues confirmed that the extruded formulations of PRP/L100 demonstrated better taste masking compared to those of PRP/ Acryl-EZE. However, each e-tongue interpreted different extent of taste masking efficiency. The e-tongues evaluation suggests that results should be cautiously considered in comparison to panellist's scores. The NMR and FT-IR analysis confirmed possible drug/polymer intermolecular interaction which could explain the mechanism underlying the taste suppression in all extruded formulations.

Acknowledgements

The authors would like to thank Attila Aranyos, Marion Bonnefille (Alpha MOS, France) and Dr Massaki Habara and Teraoka Makoto (INSENT, Japan) for their support to run the *in vitro* taste assessment studies.

Conflict of interest

The authors report no conflicts of interest.

Abbreviations

Active pharmaceutical ingredient, API; Differential Scanning Calorimetry, DSC; Dispersion Index, DI; Eudragit L100, L100; European Medicines Agency, EMA; Fourier Transform Infra-Red, FT-IR; Food and Drug Administration, FDA; glass transition, Tg; Hot melt extrusion, HME; Nuclear Magnetic Resonance, NMR; propranolol HCl, PRP; Partial Least Square, PLS; Principal Component Analysis, PCA; Relative standard deviation, RSD; Scanning Electron Microcopy, SEM; Standard deviation, SD.

References

1. Maniruzzaman M et al. Taste masking of paracetamol by hot-melt extrusion: an in vitro and in vivo evaluation. *Eur J Pharm and Biopharm* 2012; 80(2): 433-42.
2. Gryczke A et al. Development and evaluation of orally disintegrating tablets (ODTs) containing ibuprofen granules prepared by hot melt extrusion. *Coll and Surface B Biointerfaces* 2011; 86(2): 275-84.
3. Ley JP Masking bitter taste bymolecules, *Chemosensory Perception* 2008; 1:58–77.
4. Mennella J, Beauchamp G.Optimizing oral medication for children, *Clinical Therapeutics* 2009; 30: 2120–2132.
5. Regulation (EC) No 1901/2006 of the European Parliament and of the Council of 12 December (2006) on Medicinal Products for Paediatric Use.
6. Maniruzzaman M et al. Drug-polymer intermolecular interactions in hot-melt extruded solid dispersions. *Int J Pharm* 2013; 443(1-2): 199-208.
7. Cram A. Challenges of developing palatable oral paediatric formulations, Int J Pharm 2009; 365: 1–3.
8. EMA,2007.(http://ec.europa.eu/enterprise/pharmaceuticals/paediatrics/docs/draft guideline pip 2007- 02.pdf). Accessed 23/10/2012.
9. Abu-Diak OA et al. Understanding the performance of melt-extruded poly(ethylene oxide)-bicalutamide solid dispersions: characterisation of microstructural properties using thermal, spectroscopic and drug release methods. *J Pharm Sci* 2012; 101(1): 200-13
10. Wilson M et al. Hot-melt extrusion technology and pharmaceutical application. *Therapeutic Delivery* 2012; 3 (6): 787-797.
11. Maniruzzaman M et al. Taste masking of bitter APIs by using hot melt extrusion (HME). *AAPS Journal* 2011 13 (S2), T3273-T3273.
12. Ciosek P et al. The recognition of growth conditions and metabolic type of plants by a potentiometric electronic tongue. *Electroanalysis* 2006a; 18: 1266–1272.

13. Ciosek P, Wróblewski W. The analysis of sensor array data with various pattern recognition techniques. *Sensors and Actuators* 2006b; B 114: 85–93.
14. Legin A et al. Electronic tongue for pharmaceutical analytics: quantification of tastes and masking effects. *Anal Bioanal Chem* 2004; 380: 36–45.
15. Ciosek P, Wróblewski W. Sensor arrays for liquid sensing–electronic tongue systems. *The Analyst* 2007; 132: 963–978.
16. Jańczyk M et al. Electronic tongue for the detection of taste-masking microencapsulation of active pharmaceutical substances. *Bioelectrochemistry* 2006; 80: 94–98.
17. Woertz K. et al. Rational development of taste masked oral liquids guided by an electronic tongue. *Int J Pharm* 2010; 400: 114–123.
18. Woertz K. et al. A comparative study on two electronic tongues for pharmaceutical formulation development. *J Pharm Biomed Anal* 2011a; 55:272–281.
19. Maniruzzaman M, Bonnefille M, Aranyos A, Snowden MJ, Douroumis D. An in-vivo and in-vitro taste masking evaluation of bitter melt-extruded drugs. *J Pharm Pharmacol* 2014; 66(2):323-37.
20. Altan S et al. An Application of Serially Balanced Designs for the Study of Known Taste Samples with the α-ASTREE ElectronicTongue. *AAPS PharmSciTech* 2014; In press.
21. Qiu S et al. Discrimination and Characterization of Strawberry Juice Based on Electronic Nose and Tongue: Comparison of Different Juice Processing Approaches by LDA, PLSR, RF, and SVM. *J Agric Food Chem* 2014;62 (27):6426-34
22. Woertz K et al. Taste sensing systems (electronic tongues) for pharmaceutical applications, *Int J Pharm* 2011b; 417: 256– 271.
23. Nair Ret al. Influence of various drugs on the glass transition temperature of poly(vinylpyrrolidone): a thermodynamic and spectroscopic investigation. *Int J Pharm* 2001; 225: 83-96.
24. Lee HK et al. Propranolol-/methacrylic acid copolymer binding interaction, *J Pharml Sci* 1991; 80:178-180.
25. Avram M, Mateescu GH. Infrared Spectroscopy: Applications in Organic Chemistry, Wiley/Interscience, New York. 1996.

26. Qi S et al. Novel characterisation of phase separation in hot melt extruded solid dispersions: a thermal, microscopic and NMR relaxometry study. *Pharm Res* 2010; 27: 1869–83.

27. Qi S et al. Characterisation of solid dispersions of paracetamol and EUDRAGIT® E prepared by hot melt extrusion using thermal, microthermal and spectroscopic analysis. *Int J Pharm* 2008; 354, (1–2):158–67.

Tables

Table 1: Sample preparation for taste masking analysis

Description	Type	Drug (%)	Polymer (%)	Drug (mg)	Polymer (mg)	Total (mg)
PRP	Active	100	0	100	0	100
Acryl-EZE	Polymer	0	90	0	900	900
PRP/ Acryl-EZE	Extrudates	10	90	100	900	1000
L100	Polymer	0	90	0	900	900
PRP/L100	Extrudates	10	90	100	900	1000

Table 2: Mean standard deviation (SD) and relative standard deviation (RSD) for each solution for *in vitro* taste analysis by Astree e-tongue.

PRP dissolution after 60s			
Formulation	**Mean SD**	**Mean RSD (%)**	**Interpretations**
PRP	8.0	0.7	Good
PRP/ **Acryl-**EZE	12.0	0.9	Good
PRP/L100	10.0	1.4	Good
Acryl-EZE	13.0	1.1	Good
L100	8.0	0.8	Good
AVERAGE	13.0	0.7	Good

$$RSD = \frac{SD}{\bar{x}} x\,100$$

561 **Figures Caption List**

562

Fig. 1 SEM images of PRP/polymer extrudates and particle size distribution.

Fig. 2 DSC thermal transitions of (i) PRP and polymers pure, (ii) PRP/polymer extruded formulations.

Fig. 3 Sensory scores of all formulations by panelist (n=6).

Fig. 4a Signal comparison between active and placebo formulations with L100 and Acryl-EZE and PRP (dissolution for 60s).

Fig. 4b Distance and discrimination comparison between signal of PRP pure and their formulations on Astree e-tongue (after 60s dissolution).

Fig. 4c Sensory correlation model based on PLS with Astree e-tongue

Fig. 5a Normalised DI (%) of all drug/L100 formulations in four different time scale.

Fig. 5b Normalised DI (%) of all drug/ Acryl-EZE formulations in four different time scale.

Fig. 5c Relationship between results of taste sensors and human taste scores for similar tastes. The standard deviations on the x- and y-axes are the difference between the panelists' scores and measurement error (n = 6), respectively.

Fig. 5d Sensor output of the two polymers, Acryl-EZE and L100. Maximum concentrations of polymers were set at 60 times the maximum API conc. (approx. 0.5 mg/mL x 60 = 30 mg/mL).

Fig. 6 FT-IR spectra of PRP extruded formulations.

Fig. 7 Part, ^{1}H T1 spectra (aromatic region) for the PRP pure and taste masked formulations.

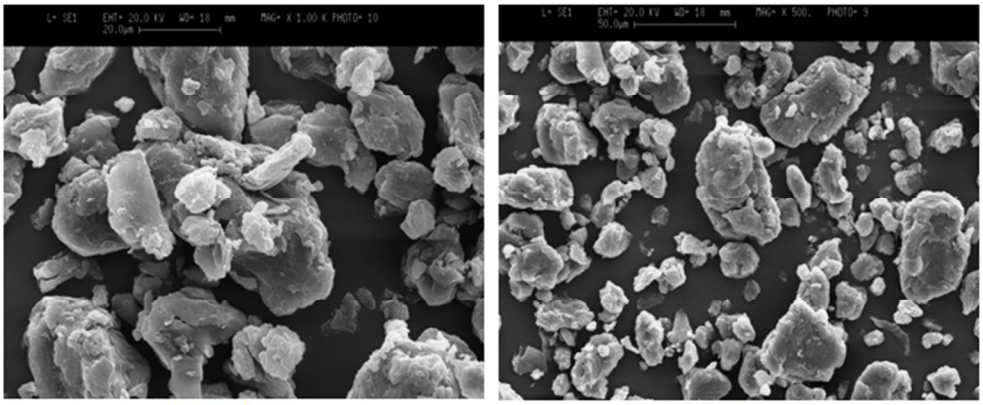
563

564

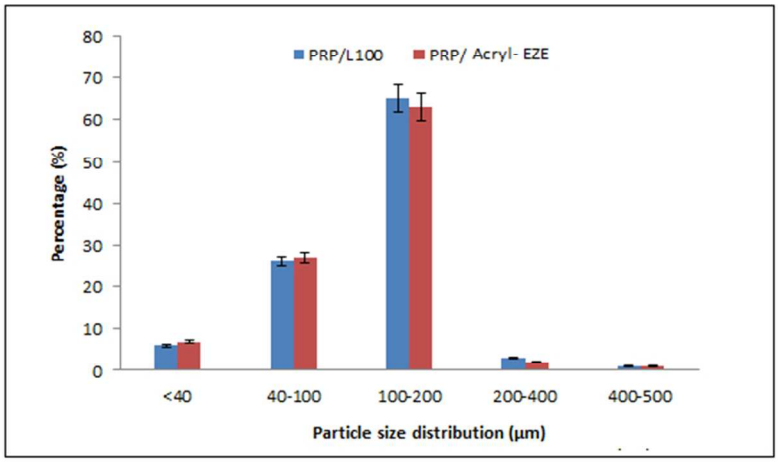
565

566

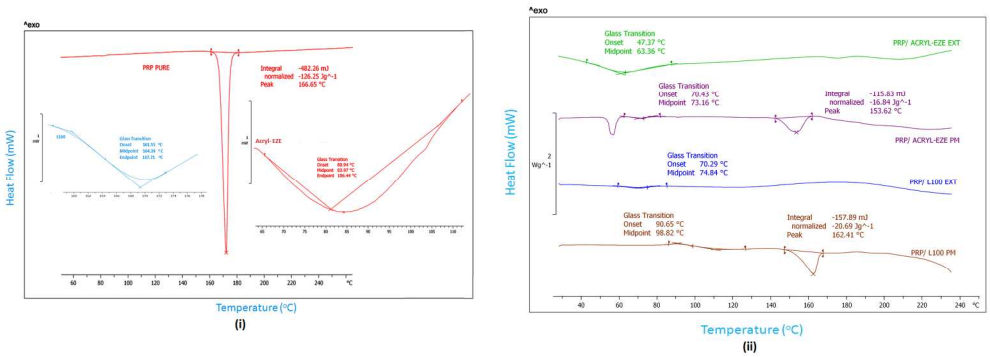
567



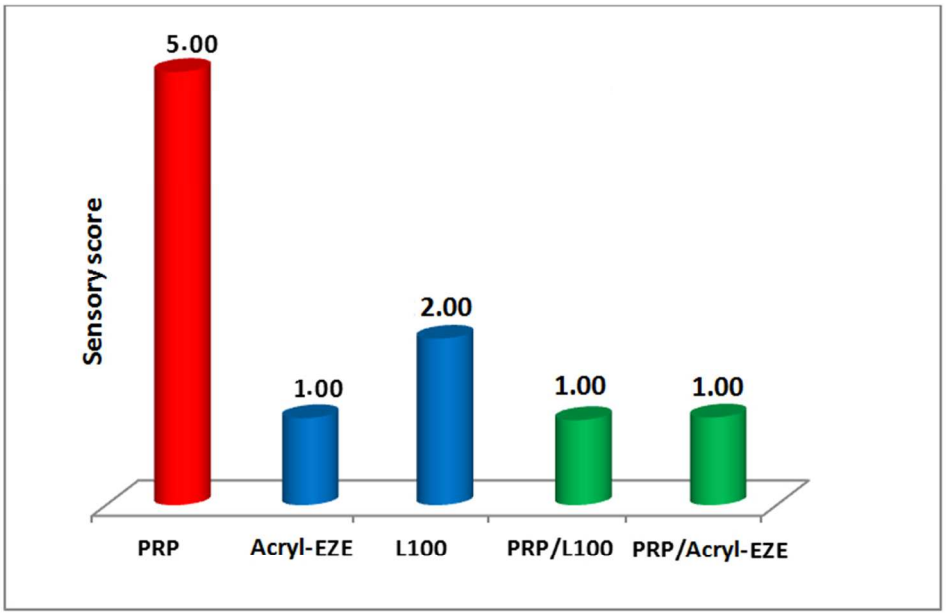
(i) PRP/L100 (ii) PRP/ Acryl-EZE



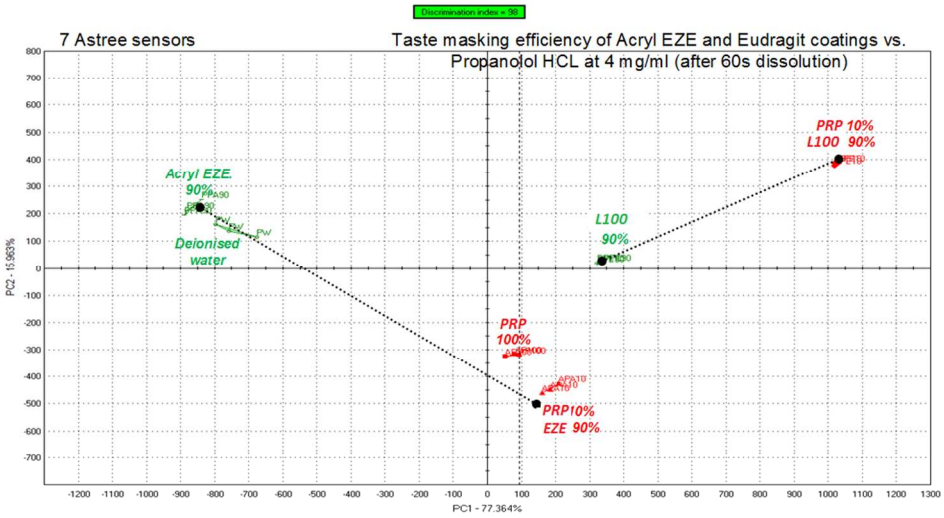
184x170mm (96 x 96 DPI)



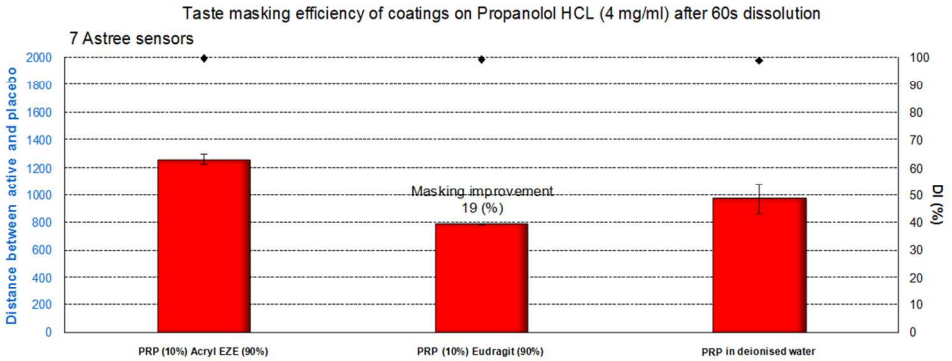
599x211mm (96 x 96 DPI)



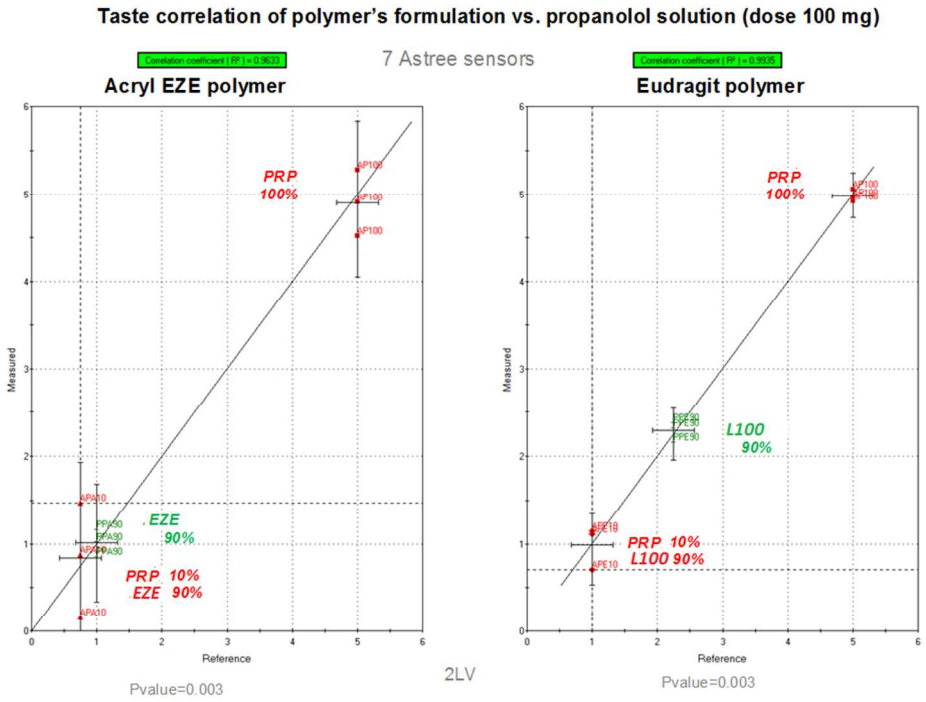
213x138mm (96 x 96 DPI)



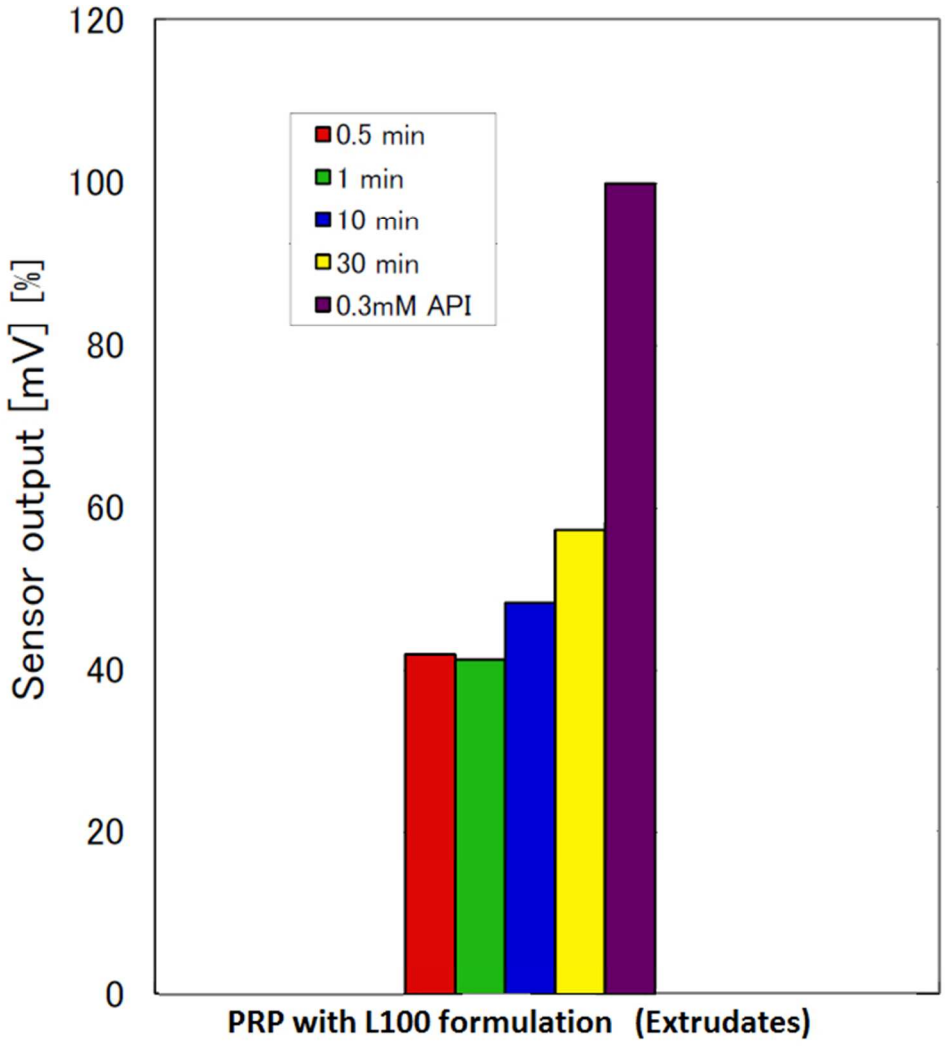
311x174mm (96 x 96 DPI)



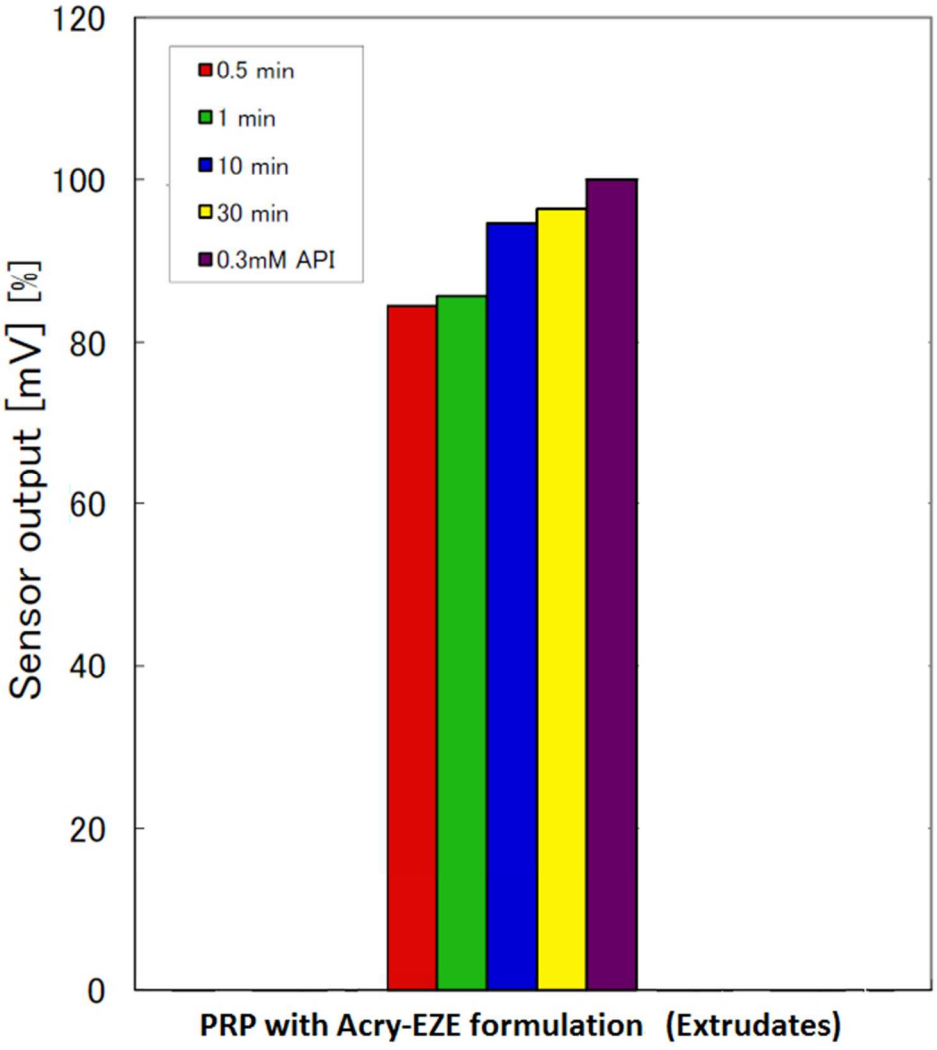
318x131mm (96 x 96 DPI)



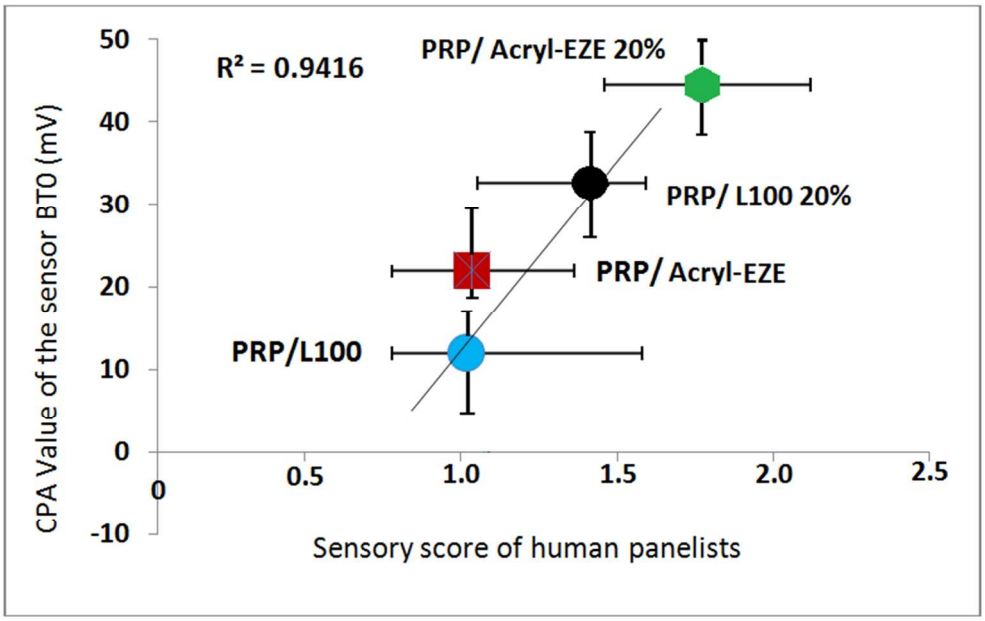
263x194mm (96 x 96 DPI)



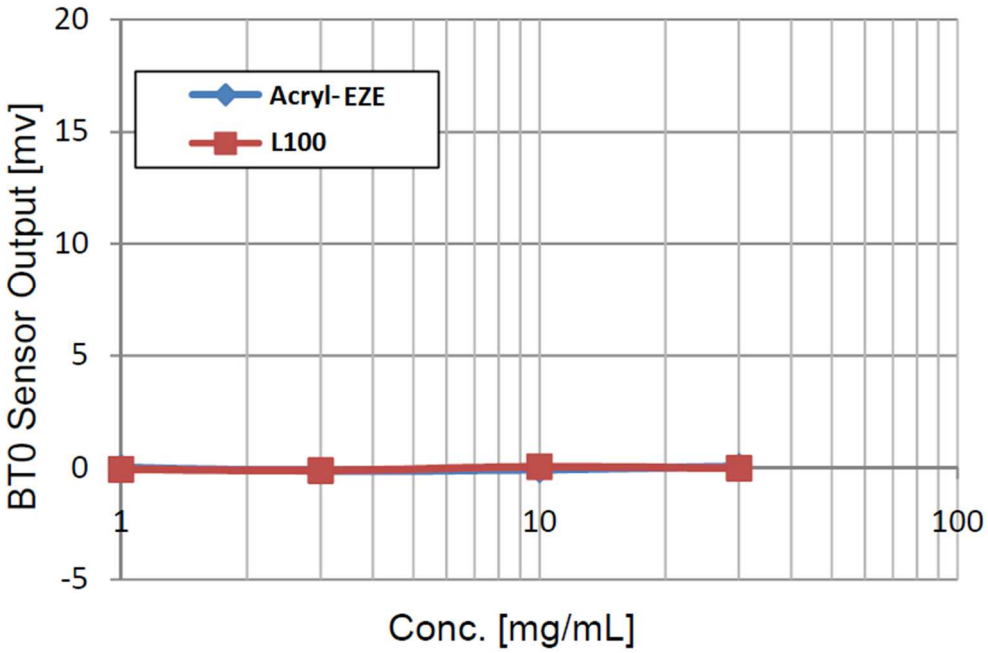
183x195mm (96 x 96 DPI)



183x201mm (96 x 96 DPI)

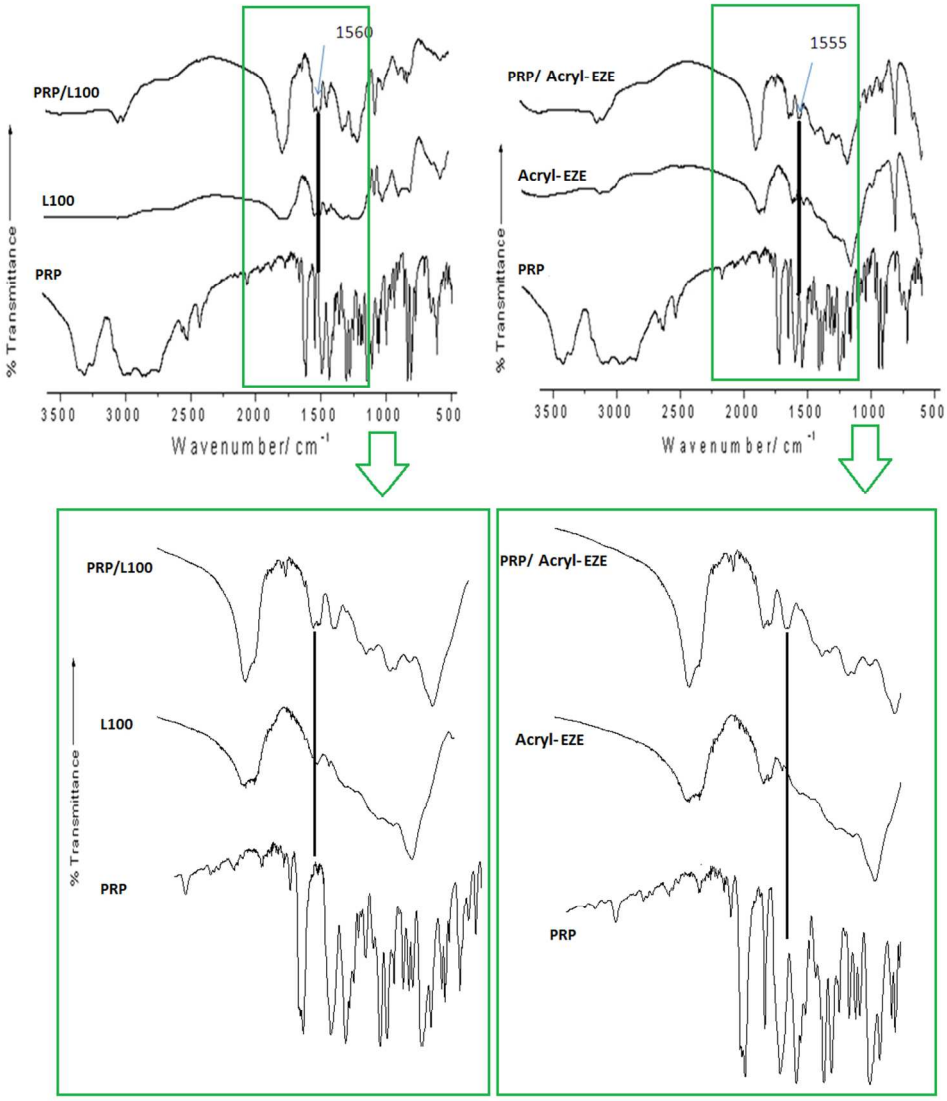


210x132mm (96 x 96 DPI)

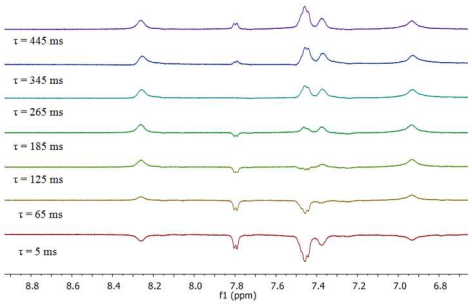


mg/mL	Acryl-EZE (mV)	L100 (mV)
1	0.01	-0.06
3	-0.14	-0.12
10	-0.08	0.05
30	0.07	-0.01

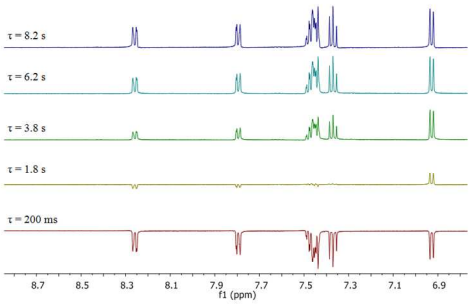
218x203mm (96 x 96 DPI)



308x351mm (96 x 96 DPI)



Part, ^{1}H T1 spectra (aromatic region) for the PRP/L100



Part, ^{1}H T1 spectra (aromatic region) for the PRP

465x166mm (96 x 96 DPI)